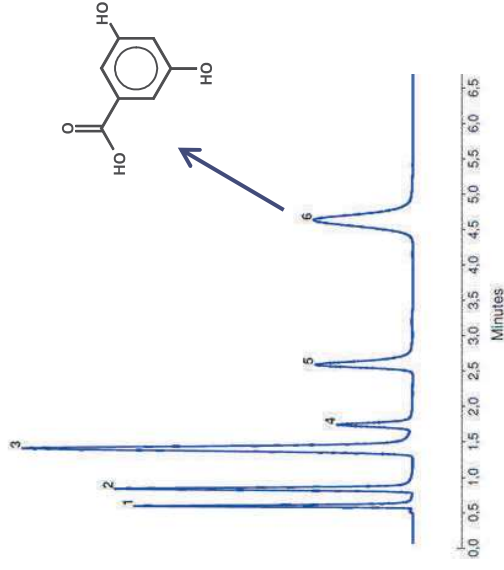


Eurospher II HILIC and Bluespher HILIC

- ▶ *How do you analyze very polar and hydrophilic compounds?*



Eurospher II HILIC and Bluespher HILIC

► What is the principle of HILIC?

HILIC or Hydrophilic Interaction Liquid Chromatography combines characteristics of the 3 major modes:

Stationary phase:

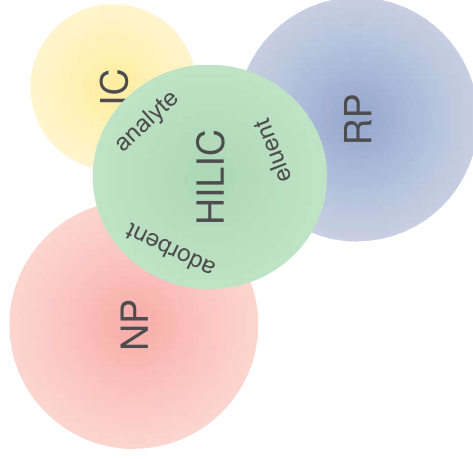
Polar modifications of silica gel or polymers - like NP-mode

Mobile phase:

Mixtures of aqueous buffer systems and organic modifiers – like RP-mode

Fields of application:

Quite polar compounds, organic and inorganic ions – like IC



Partition chromatography between mobile and stationary phase:

Distribution of analytes between water-poor layer of mobile phase and water-rich layer on surface of polar stationary phase
Weak electrostatic mechanisms and hydrogen bonds between neutral polar molecules under high organic elution

Eurospher II HILIC and Bluespher HILIC

► What are the characteristics?

Modification	% Carbon	pH range	Column code
Bluespher HILIC	7 %	2 – 8.5	XXXE120BSF
Eurospher II HILIC	7 %	2 – 8.5	XXXE120E2J

Silica gel:

ultra pure, > 99.99 %

Modification:

Ammonium – sulfonic acid

Metal content:

< 10 ppm

Particle size:

2 µm (Bluespher), 3 µm, 5 µm

Particle shape:

spherical

Pore size:

100 Å

Specific surface:

320 ± 20 m²/g

Pore volume:

0.8 ml/g

Density:

430 g/l

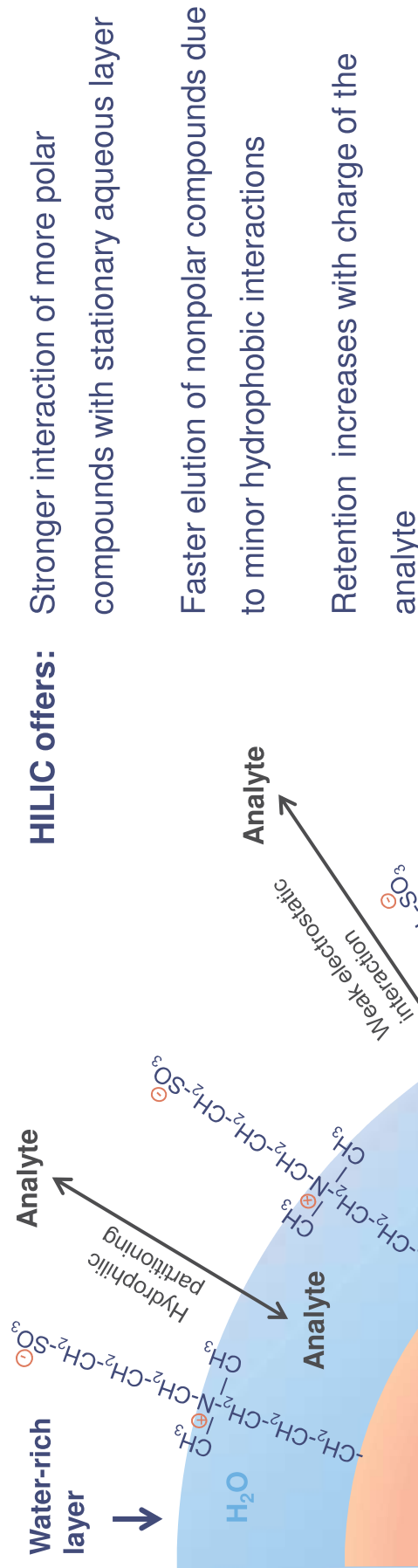


**Special zwitterionic modification:
Neutrally charged but highly polar
surface**



Eurosphere II HILIC and Bluespher HILIC

► Suffering from poor retention of polar analytes?



Elution order is often inverse on HILIC compared to RP columns

Weak electrostatic interaction → Analyte



Eurospher II HILIC and Bluespher HILIC

▶ *When are the new HILIC phases recommended?*

Recommended for the analysis of hydrophilic compounds like:

- Polar organic acids and bases
 - Natural polar compounds
 - Nucleosides
 - Oligonucleotides
 - Amino acids
 - Peptides
 - Water soluble vitamins
- ▶ Completely MS compatible
 - ▶ Ultra fast equilibration
 - ▶ Suitable in Analytical and Preparative scale



check www.knauer.net for Applications!

Application with Eurospher II HILIC

► Hydroxybenzoic acids

Eurospher II 100-5 HILIC, 150 x 3 mm ID

System: AZURA compact

Eluent: A: 5 mM NH₄-acetate pH 4.78

B: Acetonitrile

Gradient: Isocratic 90 % B

Flow rate: 1.0 ml/min

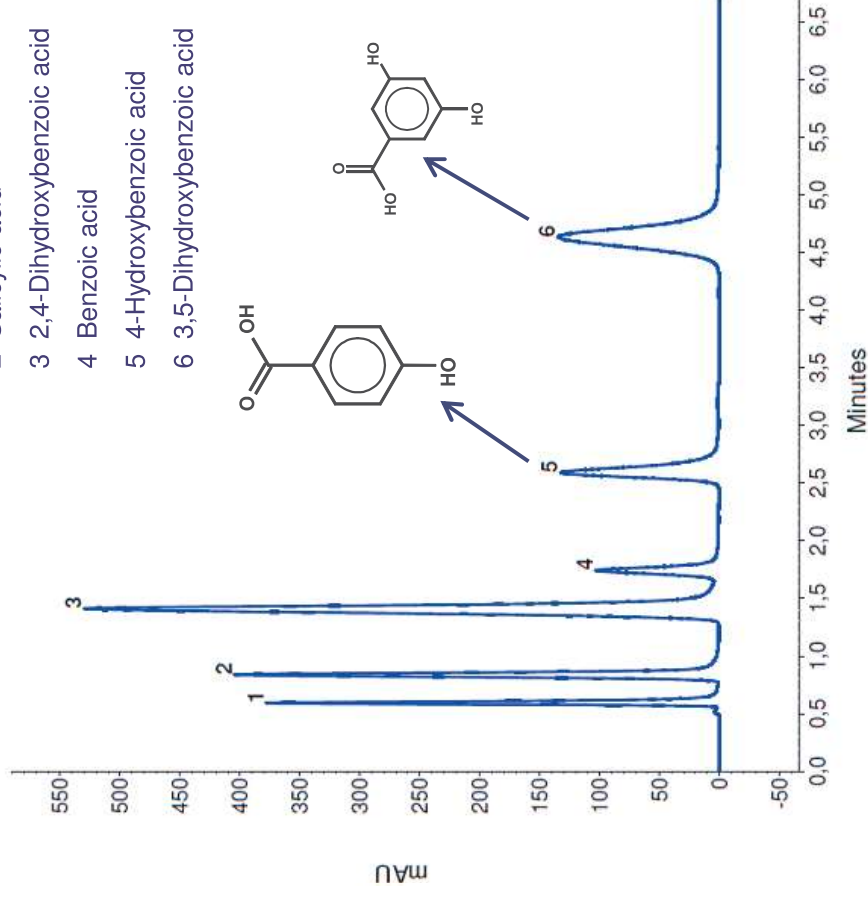
Temperature: 25 ° C

Volume: 1 µl

Detection: UVD 2.1L, 10 mm flow cell

210 nm, 20 Hz, 0,05 s

- 1 2,6-Dihydroxybenzoic acid
- 2 Salicylic acid
- 3 2,4-Dihydroxybenzoic acid
- 4 Benzoic acid
- 5 4-Hydroxybenzoic acid
- 6 3,5-Dihydroxybenzoic acid





Application with Eurospher II HILIC

► Water-soluble Vitamins

Eurospher II 100-5 HILIC, 150 x 3 mm ID

System: AZURA compact

Eluent: A: 25 mM NH₄-acetate pH 4

B: Acetonitrile

Gradient: 0.0 – 0.7 min 20 % A

0.7 – 1.4 min 20 – 30 % A

1.4 – 5.0 min 30 % A

Flow rate: 1.0 ml/min

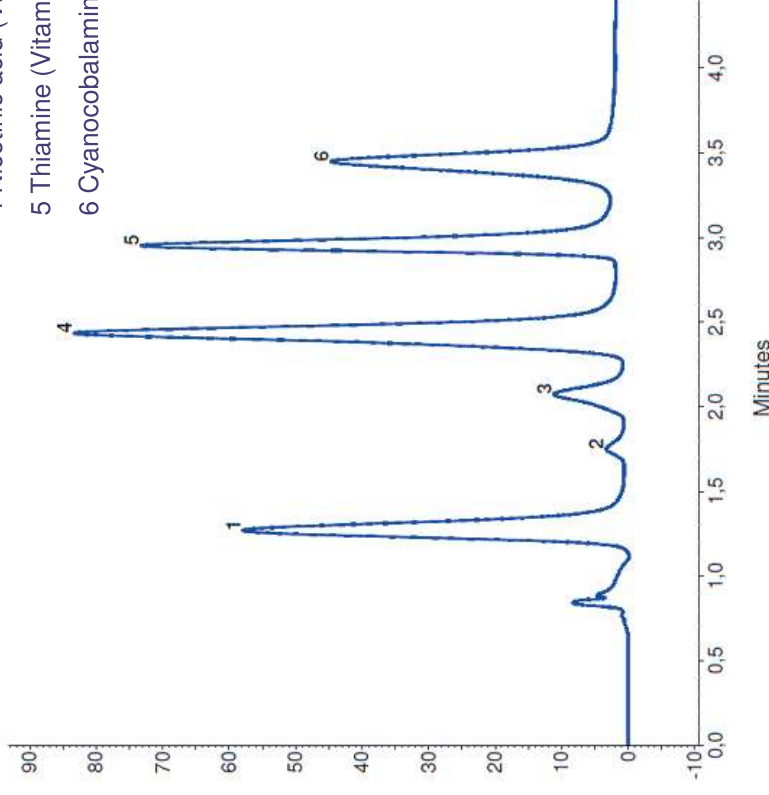
Temperature: 25 ° C

Volume: 10 µl

Detection: UVD 2.1L, 10 mm flow cell

254 nm, 20 Hz, 0,5s

- 1 Pyridoxal (Vitamin B₆)
- 2 Folic acid (Vitamin B₉)
- 3 Ascorbic acid (Vitamin C)
- 4 Nicotinic acid (Vitamin B₃)
- 5 Thiamine (Vitamin B₁)
- 6 Cyanocobalamin (Vitamin B₁₂)



Application with Eurospher II HILIC

► Organic acids

Eurospher II 100-5 HILIC, 150 x 3 mm ID

System: AZURA compact

Eluent: A: 200 mM NH₄-acetate pH 6.8

B: Acetonitrile

Gradient: Isocratic 70 % B

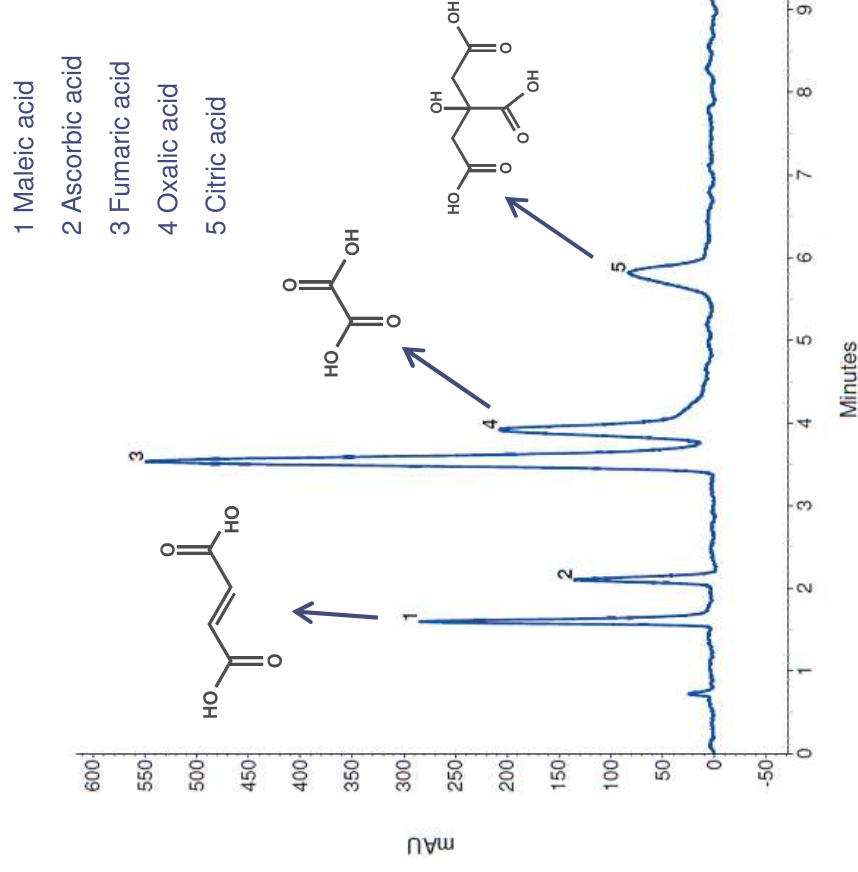
Flow rate: 1.0 ml/min

Temperature: 25 ° C

Volume: 1 µl

Detection: UVD 2.1L, 10 mm flow cell

210 nm, 10 Hz, 0,2s



Eurospher II HILIC and Bluespher HILIC

► Ordering Information

The last 7 digits of the Order No. comprise the stationary phase

Bluespher* 100-2 HILIC	Order No. ...E120BSF
Eurospher II 100-3 HILIC	Order No. ...E120E2G
Eurospher II 100-5 HILIC	Order No. ...E120E2J

*Only available in 2 mm ID columns.

The first 3 digits of the Order No. comprise the column dimensions

analytical columns ID	2 mm ID	3 mm ID	4 mm ID	4.6 mm ID	8 mm ID
5 mm (pre column)	P5B...	P5C...	P5D...	P5D...	n.a.
30 mm length	03B...	03C...	03D...	03D...	03G...
50 mm length	05B...	05C...	05D...	05D...	n.a.
60 mm length	06B...	06C...	06D...	06D...	06G...
100 mm length	10B...	10C...	10D...	10E...	n.a.
120 mm length	11B...	11C...	11D...	11E...	11G...
125 mm length	12B...	12C...	12D...	12E...	n.a.
150 mm length	15B...	15C...	15D...	15E...	n.a.
250 mm length	25B...	25C...	25D...	25E...	25G...
300 mm length	n.a.	n.a.	30D...	n.a.	30G...

